ABSTRACT

Alpinia and *Kaempferia* are two important genera of the family Zingiberaceae, possessing significant medicinal and aromatic properties. In spite of the great economical potential of the species of *Kaempferia* and *Alpinia*, they have received much less attention from biotechnologists for their phytochemical profiling, molecular characterization and conservation. The plant extracts and essential oils of these species possessing myriad of therapeutic activities, are of high demand in pharmaceutical industries. Besides, the important bioactive constituents like ethyl-p-methoxy cinnamate in *Kaempferia galanga*, 1,8-Cineol in *Alpinia galanga* have tremendous export potential. Due to overexploitation of these wild medicinal plants, there is rising alarm about declining populations, loss of genetic diversity and habitat deprivation. Therefore agencies concerned with conservation policies like BSI, ENVIS (MoEF) etc have recommended for domestic cultivation of these wild species as a viable alternative to rise above the problems that are inherent in herbal extract. Thus it is essential to develop phytochemical profiles which represent the bioactive constituents of the herbal drugs in order to find out the elite genotypes with high drug yielding potential. Further, the interest in natural products has necessitated the search for alternate sources of natural antioxidant, antimicrobial and anticancer agents. Thus the present study aiming at phytochemical evaluation of 7 target species such as *Alpinia galanga*, *Alpinia malaccensis*, *Alpinia nigra*, *Alpinia calcarata*, *Kaempferia galanga*, *Kaempferia rotunda* and *Kaempferia parishii* assumes significance.

In spite of vast economic potential and rich diversity among the species of *Kaempferia* and *Alpinia* in India, very little work has been done on their molecular characterization. Traditionally, the species characterization has been made on the source of the morphological markers. The accessibility of small figure of morphological markers, their weakly recognized genetic control and environmental influence on phenotypic expression at diverse stages of development are a few of the established impediment in using these as steady genetic markers. Moreover identification of taxa through herbarium is very difficult because of problem in preserving thick and fleshy rhizomes. The precise molecular characterization of available germplasm using molecular markers is one step ahead providing precise genetic information meant for proper identification and improvement of the desired taxa. DNA-based molecular markers have a great utility

for the characterization of medicinally important plant species. The polymerase chain reaction (PCR) based technologies such as RAPD, ISSR, SSRs and other sequence based markers such as matK, rbcL etc are widely appreciated for its genetic integrity and simplicity. These molecular markers can provide a fast and reproducible identification tool for several taxa. Thus the present study is designed for molecular profiling of all species of *Alpinia* and *Kaempferia*.

Due to the growing demand for plant parts like root, stem, leaf, rhizome and whole plant of most of the Zingiberaceous species, they are severely threatened. The indiscriminate collection of plants coupled with vanishing forests and grasslands has resulted the depletion of gingers in the wild. As medicinal plants are constantly under threat because of over exploitation and depletion of natural habitats, the need for their rapid multiplication and conservation have gained importance. Tissue culture techniques contribute a vital role for the conservation of vegetatively propagated Zingiberaceous species as a substitute to conventional field genebanks to preserve against pests and environmental vagaries. Regeneration and successful propagation of genetically stable plantlets from culture are fundamentals for any *in vitro* conservation attempt. The present work aims at a suitable protocol for true-to-type *in vitro* production of selected plant species with high drug yielding potential and effort has been made to ensure desired genetic stability of *in vitro* propagated plantlets for more than 3 years by analyzing them with standardized molecular markers.

Phytochemical evaluation was carried out through GC-MS based phytochemical analysis of essential oil and extracts, phytochemical screening, evaluation of total phenolic and flavonoid contents as well as their bioactivity studies.

GC-MS analysis of leaf oil revealed that eucalyptol (31.10±0.4%), α-phellandrene (43.90± 0.5%) and β-pinene (56.27±2.5%) comprised maximum peak area in *A. galanga, A. malaccensis* and *A. nigra* respectively. Rhizome oil contained highest percentage of eucalyptol (36.04±0.5%), α-phellandrene (26.59±0.5%), β-pinene (38.03± 0.25%), ethyl p-methoxy cinnamate (82.01±0.25%) and benzoic acid (58.27±0.45%) in species of *A. galanga*, *A. malaccensis*, *A. nigra*, *K. galanga* and *K. rotunda* respectively.Leaf extract comprised highest percentage of benzenepropanal (37.35±0.5%), acetylcyclopentanone (9.86±0.3%), α-caryophyllene(16.75±0.3%), olealdehyde (32.41±0.95%), 2-(3,4-dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-

chromen-4-one (18.26±0.35%), phytol (93.32±0.75%) and phytol (72.55±0.5%) in species of *A. galanga, A. malaccensis, A. nigra, A. calcarata, K. galanga, K. rotunda* and *K. parishii* respectively whereas rhizome extract of these species contained maximum percentage of carotol (17.44±0.3%), 5-hydroxymethylfurfural (36.11±0.4%), pyranone (24.35±0.4%), hydroquinone (44.17±0.36%), ethyl p-methoxycinnamate (80.39±0.85%), vinylacetic acid (52.40±0.56%) and totarol (74.96±0.86%) respectively.

Phytochemical screening carried out revealing presence of alkaloids, flavonoids, steroids, triterpenoids in all the seven species indicating possible bioactive potential of these plants. Evaluation of total phenolic content (TPC) of leaf and rhizome extracts of *Alpinia* and *Kaempferia* species revealed that among the seven species screened, leaves of *A. galanga* had the highest TPC value i.e. 77.25±1.56 mg GAE/g of the extract while rhizome of *K. parishii* exhibited lowest TPC value i.e. 27±0.36 mg GAE/g of the extract. Likewise total flavonoid content (TFC) was found highest (78.84±0.81mg Quercetin equivalent/g of the extract) in *A. nigra* leaf extract while rhizome of *K. parishii* had lowest TFC value i.e. 27.30±0.43 mg Quercetin equivalent/g of the extract.

Antioxidant potential of the essential oils and extracts obtained from leaves and rhizomes of *Alpinia and Kaempferia* species were evaluated using DPPH free radical scavenging assay. Among all the essential oil and extract samples, leaf oil of *Alpinia nigra* exhibited highest antioxidant activity with lowest IC_{50} value 4µg/ml while rhizome extract of *Alpinia calcarata* showed lowest antioxidant activity (IC_{50} value:103µg/ml).

Antimicrobial activity of essential oil and extract samples of *Alpinia and Kaempferia* species was evaluated by IZD, MIC and MBC values. Among all samples the IZD value was found highest (25.63±0.72mm against *C. albicans*) in *A. nigra* leaf extract whereas it was lowest (1.7±0.5mm against *A. baumannii*) in *K. parishii* leaf extract. MIC was highest (0.55mg/ml) in *A. malaccensis* leaf extract against *S.aureus* and it was lowest (21.96mg/ml) in *K. parishii* leaf extract against *E.coli*. MBC was highest (1.51mg/ml) in *A. galanga* rhizome extract against *S.aureus* and was lowest (28.35mg/ml) in *K. galanga* leaf extract against *A.niger*.

Essential oils of *Alpinia* and *Kaempferia* species have been screened for anticancerous activity against two cell lines i.e. HeLa and MCF7 by MTT assay. In case of HeLa cell

line, at 20µg/ml concentration, the rhizome essential oil of *K. galanga* displayed highest (82.33% ± 3.76) percentage of inhibition followed by *A. nigra* rhizome oil (79 %± 2.6) and *A. malaccensis* leaf oil showed lowest (14.55% ± 0.52) percentage of inhibition. Among the oils tested on MCF7 cell line, *K. galanga* rhizome oil showed highest (78.4% ± 4.35) percentage of inhibition followed by *A. nigra* rhizome oil (70.9% ± 5.34) and lowest inhibition (15.15% ± 0.54)was shown by *A. galanga* leaf oil.

Thus the identified important constituents of different species may be used as biomarker for development of chemical fingerprint of respective plant species for authentic identification and quality control of herbal drug. These potential chemical constituents might render the plants with high antioxidant, antimicrobial and anticancer activities. The essential oils, extracts and the chemical constituents of *Alpinia* and *Kaempferia* species with high bioactivity potential may be used for formulation for novel drugs.

Molecular characterization carried out with RAPD, ISSR, SSR and other sequence based markers revealed species specific and reproducible DNA banding pattern in all seven species. The unique bands were found in *Alpinia nigra* and *Alpinia calcarata.* These findings would be useful for authentic identification of species and for the future improvement of these taxa.

In the present study, *in vitro* culture methods have been standardised for selected taxa such as *A. galanga*, *A. malaccensis*, *K. galanga* and *K. rotunda*. For *A. galanga* MS medium fortified with BA (3 mg/L) in combination with Kn (3 mg/L) and NAA (1 mg/L) was found optimum for shoot multiplication whereas MS liquid medium with same hormone combination was suitable for *A. malaccensis*. BA (1 mg/l) with IAA (0.5 mg/l) was found optimum for shoot multiplication in *K. galanga* and in case of *K. rotunda* BA (3 mg/l) with IAA (1 mg/l) was found optimum. Molecular marker (RAPD and ISSR) based assessment of field grown micropropagated plantlets revealed genetic stability up to 3 years. The protocol of micropropagation established for 4 species has got significance for commercial production of true to type plants for stable supply of drug to market.